

## WHAT IS CLAIMED IS:

1. A crystal formed by IGF-1 that diffracts x-ray radiation to produce a diffraction pattern representing the three-dimensional structure of the IGF-1.
- 5 2. The crystal of claim 1 having approximately the following cell constants  $a=31.831 \text{ \AA}$ ,  $b=71.055 \text{ \AA}$ ,  $c=65.995 \text{ \AA}$ , and a space group of C222<sub>1</sub>.
3. The crystal of claim 1 wherein the IGF-1 contains an A-, B-, C-, and D-region and forms a dimer in the crystal and wherein the crystal comprises a receptor binding site at the dimer interface.
4. A composition comprising the crystal of claim 1 and a carrier.
- 10 5. The composition of claim 4 wherein the IGF-1 is biologically active when resolubilized.
6. A method of treating a mammal suffering from an agonist disorder, said method comprising administering to said mammal an effective amount of the composition of claim 5.
7. The method of claim 6 wherein the mammal is human.
8. The method of claim 6 wherein the disorder is diabetes, obesity, a heart dysfunction, AIDS-related wasting, a kidney disorder, a neurological disorder, a whole body growth disorder, or an immunological disorder.
- 15 9. A method of crystallizing IGF-1 comprising the steps of:
  - (a) mixing an aqueous solution comprising IGF-1 with a reservoir solution comprising a precipitant to form a mixed volume; and
  - 20 (b) crystallizing the mixed volume.
10. The method of claim 9 wherein the IGF-1 is obtained from a prokaryotic cell.
11. The method of claim 9 wherein the aqueous solution of step (a) contains about 1 to 50 mg per ml of IGF-1.
12. The method of claim 9 wherein the aqueous solution of step (a) contains about 5 to 15 mg per ml of IGF-1.
13. The method of claim 9 wherein the precipitant is polyethylene glycol, sodium citrate, ammonium sulfate, sodium cacodylate, or a mixture thereof.
14. The method of claim 12 wherein the precipitant is polyethylene glycol buffered with sodium citrate or sodium cacodylate.
- 15 15. The method of claim 13 wherein the precipitant is present in the reservoir solution in an amount of about 20 to 25% if polyethylene glycol, and about 1 to 10 M if sodium citrate, ammonium sulfate, or sodium cacodylate.
16. The method of claim 9 wherein the reservoir solution further comprises a detergent.
17. The method of claim 16 wherein the detergent is present in an amount of about 10 to 50 mM.
18. The method of claim 16 wherein the detergent is N, N-bis(3-D-gluconamidopropyl)-deoxycholate.
19. The method of claim 9 wherein the pH of the reservoir solution is about 4 to 10.
20. The method of claim 9 wherein the pH is about 6.5.
21. The method of claim 9 wherein step (b) is carried out by vapor diffusion crystallization, batch crystallization, liquid bridge crystallization, or dialysis crystallization.

22. The method of claim 9 wherein step (b) is carried out by vapor diffusion crystallization.
23. The method of claim 9 further comprising recrystallizing the IGF-1 after step (b).
24. The method of claim 23 wherein the recrystallization takes place using methyl pentanediol.
25. The method of claim 9 further comprising isolating the crystalline IGF-1.
- 5 26. The method of claim 9 wherein the aqueous solution is mixed with about 24% polyethylene glycol buffered to about pH 6.5 with either about 0.1M sodium citrate or about 0.1M sodium cacodylate and about 1  $\mu$ l of about 1.4 mM N, N-bis(3-D-gluconamidopropyl)-deoxycholamine detergent, this solution is equilibrated by vapor diffusion crystallization with about 1 mL of about 24% polyethylene glycol buffered to about pH 6.5 with either about 0.1M sodium citrate or about 0.1M sodium cacodylate until
- 10 crystallization droplets are formed, and about 2  $\mu$ l of about 100% methyl pentanediol are added to the crystallization droplets so as to dissolve the crystals overnight and thereby form new crystals.
27. Crystalline IGF-1 produced by the method of claim 9.
28. A method of identifying indirect agonists of IGF-1 comprising the steps of:
- 15 (a) comparing the ability of N, N-bis(3-D-gluconamidopropyl)-deoxycholamine to inhibit binding of IGFBP-1 or -3 to IGF-1 with the ability of a candidate indirect agonist of IGF-1 to so inhibit binding; and
- (b) determining whether the candidate agonist inhibits such binding at least as well as N, N-bis(3-D-gluconamidopropyl)-deoxycholamine.
29. The method of claim 28 wherein the comparison is accomplished by competition assay between N, N-bis(3-D-gluconamidopropyl)-deoxycholamine and the candidate agonist.
30. The method of claim 28 wherein inhibition of binding is measured by pre-incubating N, N-bis(3-D-gluconamidopropyl)-deoxycholamine or the candidate agonist with IGF-1 expressed on bacteriophage particles and measuring residual binding of IGF-1 to IGFBP-1 or IGFBP-3 in a plate-based ELISA assay.
- 25 31. A method of identifying indirect agonists of IGF-1 comprising co-crystallizing a candidate indirect agonist of IGF-1 with IGF-1 to form a co-crystalline structure and determining if the candidate agonist binds to one or both of two patches on IGF-1, wherein one patch has the amino acid residues Glu 3, Thr 4, Leu 5, Asp 12, Ala 13, Phe 16, Val 17, Cys 47, Ser 51, Cys 52, Asp 53, Leu 54, and Leu 57, and the second patch has the amino acid residues Val 11, Gln 15, Phe 23, Phe 25, Asn 26, Val 44, Phe
- 30 49, and Arg 55, and wherein binding occurs if there is at least one contact between each listed amino acid residue of a given patch and the candidate agonist that is less than or equal to 6 angstroms in the co-crystalline structure.
32. The method of claim 31 wherein the candidate agonist inhibits binding of IGFBP-1 or -3 to IGF-1 at least as well as N, N-bis(3-D-gluconamidopropyl)-deoxycholamine.
- 35 33. The method of claim 32 wherein inhibition of binding is measured using a competition assay between N, N-bis(3-D-gluconamidopropyl)-deoxycholamine and the candidate agonist.
34. The method of claim 33 wherein inhibition of binding is measured by pre-incubating N, N-bis(3-D-gluconamidopropyl)-deoxycholamine or the candidate agonist with IGF-1 expressed on bacteriophage particles and measuring residual binding of IGF-1 to IGFBP-1 or IGFBP-3 in a plate-based ELISA assay.
- 40

35. A co-crystalline complex of IGF-1 and N, N-bis(3-D-glucanamidopropyl)-deoxycholamine.
36. A method for determining a three-dimensional structure of IGF-1 comprising:
- (a) crystallizing the IGF-1;
  - (b) irradiating the crystalline IGF-1 to obtain a diffraction pattern characteristic of the crystalline IGF-1; and
  - (c) transforming the diffraction pattern into the three-dimensional structure of the IGF-1.
37. A machine-readable data storage medium comprising a data storage material encoded with machine-readable data that, when read by an appropriate machine, displays a three-dimensional representation of a crystal of a molecule comprising IGF-1.
38. An IGF-1 crystal with the structural coordinates shown in Appendix 1.
39. A method of using a three-dimensional structure of IGF-1 derived from an IGF-1 crystal wherein the three-dimensional structure of IGF-1 includes an IGF-1 receptor-binding region, the method comprising identifying compounds having structures that interact with the receptor-binding region of the three-dimensional structure of IGF-1 and function as an IGF-1 agonist or antagonist.
40. The method of claim 39 wherein the three-dimensional structure of IGF-1 includes alpha-carbon coordinates substantially the same as those of the structural information presented in Appendix 1.
41. A method of identifying IGF-1 agonists or antagonists comprising the steps of:
- (a) crystallizing IGF-1 to form IGF-1 crystals, the IGF-1 crystals containing a group of amino acid residues defining an IGF-1 receptor-binding region;
  - (b) irradiating the IGF-1 crystals from step (a) to obtain a diffraction pattern of the IGF-1 crystals;
  - (c) determining a three-dimensional structure of IGF-1 from the diffraction pattern, the structure including an IGF-1 receptor-binding region; and
  - (d) identifying an IGF-1 agonist or antagonist having a three-dimensional structure that functionally duplicates essential IGF receptor-binding, solvent-accessible residues presenting the three-dimensional structure of the IGF-1 receptor-binding region, said IGF-1 agonist or antagonist having altered signal transduction capacity to IGF-1-responsive cells, as compared to IGF-1.
42. The method of claim 41 wherein the solvent-accessible residues do not participate in formation of the IGF-1 interface.
43. A method of designing a compound that mimics the 3-dimensional surface structure of IGF-1 comprising the steps of:
- (a) determining the 3-dimensional structure of the IGF-1; and
  - (b) designing a compound that mimics the 3-dimensional surface structure of the IGF-1.
44. A method for identifying a peptidomimetic that binds IGF-1 and blocks binding of an IGF1BP or a receptor that binds to IGF-1 comprising the steps of:
- (a) searching a molecular structure database with the structural parameters or structural coordinates provided in Appendix 1; and
  - (b) selecting a molecule from the database that mimics the structural parameters or structural coordinates of the IGF-1.
45. A method for determining at least a portion of a three-dimensional structure of a molecular complex, said complex comprising IGF-1 and said method comprising the steps of:

- (a) determining the structural coordinates of a crystal of IGF-1;
- (b) calculating phases from the structural coordinates;
- (c) calculating an electron density map from the phases obtained in step (b); and
- (d) determining the structure of at least a portion of the complex based on said electron density map.

- 5
46. The method of claim 45 wherein the structural coordinates used in step (a) are substantially the same as those described in Appendix 1 or describe substantially the same crystal as the coordinates in Appendix 1.
- 10
47. A method for evaluating the ability of a chemical entity to associate with IGF-1 or a complex thereof, the method comprising the steps of:
- (a) employing computational or experimental means to perform a fitting operation between the chemical entity and the IGF-1 or complex thereof, thereby obtaining data related to the association; and
  - (b) analyzing the data obtained in step (a) to determine the characteristics of the association
- 15
48. A chemical entity identified by the method of claim 47 wherein the entity interferes with the *in vivo* or *in vitro* association between IGF-1 and its receptor or between IGF-1 and at least one of its binding proteins, or associates with a binding site on IGF-1.
49. A heavy-atom derivative of a crystallized form of IGF-1.
- 20
50. A method of computationally or experimentally evaluating a chemical entity to obtain information about its association with a binding site of IGF-1 using a crystal of IGF-1 having the structural coordinates described in Appendix 1.